

Supplemental information:

Supplemental table 1. Comparative analysis of cell death regulated gene expression by Treg and Tcon cells. Wild type CD4⁺Foxp3-GFP⁺ Treg cells and CD4⁺Foxp3-GFP⁻ Tcon were purified from the thymus or spleen by cell sorting. Total RNA was isolated and subjected to RT-MLPA analysis ²⁹. Values represent means \pm SEM of n=4-5 independent samples per tissue type analyzed in duplicates.

Supplemental figure 1. Thymic Treg cells and SP4 thymocytes display different susceptibility to apoptosis. Wild type, *Bim*^{-/-} and *vav-Bcl-2* CD4⁺Foxp3-GFP⁺ Treg cells and CD4⁺8⁺Foxp3-GFP⁻ thymocytes were purified from the thymus and survival was analysed by AnnexinV/7AAD staining. Only AnnexinV/7AAD negative cells were considered alive. Data points represent means \pm SEM of n=3-7 animals in ≥ 3 independent experiments. Cells were cultured either (A) in medium for 9, 18 and 48h or in the presence of (B) 100 U/ml IL-2, (C) 20 ng/ml IL-7, (D) 1 μ M SAHA, (E) 100 ng/ml FasL, (F) 10⁻⁸ M Dexamethasone, (G) 10 μ g/ml Etoposide and (H) 100 nM Staurosporine for 18h. For calculation of increased and relative survival cell viability was normalized to medium values; Symbols and bars represent means \pm SEM of n=3-10 datapoints acquired in ≥ 3 independent experiments; statistics: Student's t-test * p \leq 0.05, ** p \leq 0.01, *** p \leq 0.001 Treg cells were compared to SP4 cells; + p \leq 0.05, ++ p \leq 0.01, +++ p \leq 0.001 wild type Treg and SP4 cells, respectively were compared to their *Bim*^{-/-} and *vav-Bcl-2* counterparts.

Supplemental figure 2. Reduced expression of Treg cell marker by *Bim*^{-/-} and *vav-Bcl-2* Treg cells. Representative histogram plots of wt, *Bim*^{-/-} and *vav-Bcl-2* Treg

cells from the thymus (upper panel) or spleen (lower panel) of (A) Foxp3-GFP, (B) GITR and (C) CTLA-4. Cells are gated on CD4⁺Foxp3-GFP⁺ Treg cells.

Supplemental figure 3. Inflammatory status in spleens of colitis induced mice.

(A) Absolute spleen cell number (left panel) and Tcon (middle panel) and Treg cell number (right panel) in the spleens of mice receiving wild type Tcon cells, either together with wild type (wt) or *vav-Bcl-2* (Bcl-2) Treg cells. Bars represent means \pm SEM of n=4 animals per group; statistics: Student's t-test ** $p \leq 0.01$ Representative dot plots quantifying (B) IFN- γ or (C) IL-17A production in CD4⁺Foxp3-GFP⁺ Tcon cells 5h after *in vitro* stimulation in the presence of PMA/Ionomycin.

Supplemental figure 4. Inflammatory conditions enhance Treg cell marker expression and suppressive capacity of Treg cells overexpressing Bcl-2. On day 46 after cell transfer mice were sacrificed and expression of (A) CD25 (spleen), (B) Foxp3-GFP (spleen), (C) CD4 (spleen), (D) GITR and (E) CTLA-4 (mLN and spleen) was assessed by flow cytometry on wild type and *vav-Bcl-2* Treg cells. For quantification of CD25, Foxp3-GFP, GITR and CTLA-4 expression, cells were gated on CD4⁺Foxp3-GFP⁺ and for CD4 expression on total Foxp3-GFP⁺ Treg cells. Bars represent means \pm SEM of n=4 animals per group; statistics: Student's t-test ** $p \leq 0.01$ (F) Naïve Treg cells were isolated from wild type or *vav-Bcl-2* mice. Colitic wild type or *vav-Bcl-2* derived Treg cells were purified from *RAG1*^{-/-} mice 46 days after cell co-transfer (see figure 6). Naïve wild type Tcon cells were stimulated with anti-CD3 mAb in the absence or presence of Treg cells in a 1:1 ratio for 3 days. Cell proliferation was normalized to Tcon cell proliferation in the absence of Treg cells. Bars represent means of triplicates.